Optimum Concentration *Anredera cordifolia* (Ten.) Steenis Gel in Increasing the Expression BMP-2 and the number of Osteoblasts Post Tooth Extraction in Wistar Rats

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Abstract

Healing or recovery of damaged tissue basically is a replacement of the damaged tissue with the new ones to become normal tissue. The process of tissue recovery is the first stage of dynamic processes. The healing process is important for normal structure maintenance, function, and life perpetuity of an individual, one of which has important role is fibroblast and osteoblast. *Anredera cordifolia* (Ten.) Steenis leaf is a local plant that frequently used as medicinal treatments in Indonesia was also known as binahong leaf, some of them to heal wounds, but there had never been research of the use of its leaf in wound recovery after tooth extraction especially in BMP-2 and osteoblast proliferation.

The aim of this study was to investigate the effect of *A. cordifolia* (Ten.) Steenis gel in accelerating the expression of BMP-2 and the number of Osteoblasts post tooth extraction in Wistar Rats. This study was used post test only control group design. 36 male Wistar Rats weight between 150-200 grams, 3 months of age are being used. Tooth extraction is being done on lower left incisor. The 36 rats are divided into three groups. The data were analyzed statistically using One-Way ANOVA and LSD 0.05. The result of every tested group showed $p > 0.05$, therefore all the data had a normal distribution. Therefore, a One-Way Anova test with 5% significant rate was done and continued by LSD test to find a significant difference in each groups. Examination showed there was significant difference in expression BMP-2 and the number of Osteoblasts between *A. cordifolia* (Ten.) Steenis gel 10% and two other groups ($p < 0.05$). The application of *A. cordifolia* (Ten.) Steenis gel 10% can accelerate the expression BMP-2 and the number of Osteoblasts post tooth extraction in Wistar Rats.

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Introduction

The healing process is important for normal structure maintenance, function, and life perpetuity of an individual, one of which has important role is fibroblast and osteoblast. Healing of tooth extraction is needed as soon as possible by dentists. Late wound recovery process post tooth extraction, may have some trouble to soft and hard tissues in area post extraction. This process can prevent experiences to complication in alveolar bone and gingival tissues. The use of medicine post the tooth extraction can reduce the possibility of complication and it is often expected to be able to gain the process of coagulating of blood, so that it will also have the process wound recovery soon.[1-3]

In the present study, it is known many uses of natural ingredients to help and accelerate wound recovery process. One of them is *Anredera cordifolia* (Ten.) Steenis or commonly known as binahong. Other than being used for wound recovery process, this plants also has numerous usefulness such as facilitate circulation of blood, diuretic, antipyretic, haemostatic, anti bacterial, and anti inflammatory. In tooth extraction, the process that occurs in the damaged tissue is wound healing that can be divided into several phases, which is hemostasis, inflammation, proliferation and tissue remodeling. In wounds caused by tooth extraction, there will be a repair process that includes soft tissues and hard tissues of the oral cavity. Soft tissue consists of

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gingival and periodontal ligaments, and hard tissue is the alveolar bone.[4]

Osteoblasts are mononucleated cells that synthesize collagen and non-collagen matrix. Osteoblasts originate from mesenchymal cells, producing osteoid or bone matrix, round, oval or polyhedral, separate from the matrix that has undergone mineralization. Osteoblasts have the function of being able to synthesize and secrete bone organic matrix, regulating electrolyte changes in extracellular fluid in the mineralization process. Osteoblasts contain endoplasmic reticulum, golgi apparatus and mitochondria. Osteoblast maturation requires fibroblast growth factor (FGF), bone morphogenic proteins (BMPs), core binding factor-1 (CBF-1) and osteoblast specific cis acting element (OSE-2). Osteoblasts have estrogen receptors, cytokines, parathyroid hormone (PTH), insulin derivated growth factor (IGF), and Vitamin D3. Osteoblasts are interconnected through a gap junction. Osteoblasts that settle on the bone surface are flat, called bone lining cells / resting osteoblasts. Osteoblasts secrete several BMP families such as BMP 2 and BMP 7, also transforming growth factor-β (TGF β), insulin-like growth factors (IGF-1 and IGF-II), platelet-derived growth factor (PDGF) and fibroblast growth factors (FGF).[5-6]

Wound is damaged on body tissue which is caused by a laceration or trauma which cause continuity disturbance structure. In dentistry, wound related to mucous and hard tissues commonly faced by dentists. Wound recovery is not a simple linear process, it is a complex but systematic process which involves a number of blood cell, cytokines, connective tissue, and growth factor activities. In normal circumstance, the wound recovery process pasess three stages, the first is inflammation stage where signs of inflammation exist such as reddening wound, pain and swollen. The next stage, the remodeling stage, where in this final part the wounded skin is almost as good as it was. BMP-2 and Osteoblast are some factors which have important role in proliferation phase in wound recovery process. The main function of BMP-2 is to accelerate production of alveolar bone which is produce by osteoblast.[7-8]

Anredera cordifolia (Ten.) Steenis commonly used as herbal medicinal treatments, some of them to heal mucous wounds and extraction sockets, so the opimum concentration of *A. cordifolia* (Ten.) Steenis gel to heal and accelerate bone formation after tooth extraction needs to be done. The purpose of this research is to know Optimum Concentration *A. cordifolia* (Ten.) Steenis Gel In Increasing the expression BMP-2 and the number of osteoblasts Post Tooth Extraction on Wistar Rats.

Materials and methods

This study is an experimental laboratory research using The Post-Test Only Control Group Design. 36 male Wistar Rats weigh between 150-200 grams, 3 months of age are being used. Have well condition, food and drink water given ad libitum. All animal procedures were approved by the University of Airlangga Surabaya Animal Care and Use Committee. This kind of animal is used because tooth extraction on Wistar Rats is easier with sufficiently wide socket extraction wound for applying *A. cordifolia* (Ten.) Steenis Gel. Tooth extraction is being done on lower left incisor. The choosing of lower incisor is based on the structure and anatomical form of Rat's teeth which enable extraction to be done. The 36 rats are divided into three groups. In the first and the second groups, after the extraction is done, *A. cordifolia* (Ten.) Steenis gel 5% and 10% is applied on the extraction wound. In the third group, bone graft is applied to the extraction wound as control group.

Anredera cordifolia (Ten.) Steenis leaf which is made into gel form will be easier to be put into the extraction wound socket because of its solid, soft and elastic characteristics. This gel forms makes the substance durable in extraction wound socket, so that it helps the body in wound recovery process. The making of *A. cordifolia* (Ten.) Steenis Gel is uses the mixture of HPMC and distillation of *A. cordifolia* (Ten.) Steenis leaf. The characteristics of HPMC are for thickening, stabilizer, gel maker and in some things as emulsifiers. In hydrocolloid emulsion system it doesn't function as emulsifiers, but more as substance which gives stabilization. This HPMC is easily soluble in hot or cold water, so it is easy to use. It is used as stabilizer because it's easily obtainable and also reasonably priced.[4,9]

Animal's mandible were decaputated at intervals of 3 and 7 days after extraction by median-sagittal cut, samples that had been detached from the body then fixated, Buffered isotonic solution of 10% formaldehyde was used for fixatives. 96% ethanol was used to extracted the water from the fragment. The ethanol then

replaced with a solvent miscible with the embedding medium. In paraffin embedding, the solvent used is xylene. Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven at 58-600C. The heat causes the solvent to evaporate, and the space within the tissue become filled with paraffin. The tissue together within its impregnating paraffin hardens taken out of the oven. Tissues embedded with plastic resin dehydrated in ethanol. The hard blocks containing the tissues are then taken to a microtome and sliced into thin sections 4-5μm. The sections are floated on water and transferred to glass slide to be stained. Staining tissue with Hematoksillin-Eosin and an anti-BMP-2 antibody (Novusbio, USA) was done to make the various tissue component conspicuous. Under the light microscope (Olympus, JAPAN) tissue are examined via a light beam that is transmitted through the tissue using image magnified 400 times.[4]

Results

The mean and standard deviation of the expression of BMP-2 and number of Osteoblasts post extraction in Wistar Rats is shown in the table 1 and table 2. A Kolmogorof Smirnov test was carried out on the data to determine the normality of distribution. The result of every tested group showed $p > 0.05$, therefore all the data had a normal distribution.

Therefore, a One-Way Anova test with 5% significant rate was done and continued by LSD test if there was a significant difference. The result on the 3rd and 7th day examined via a light beam that is transmitted through the tissue using image magnified 400 times shows that the expression of BMP-2 and amount of Osteblast on the group which is given *A. cordifolia* (Ten.) Steenis Gel is much more than the control group. The result shows there is significant difference in each group treatment $p < 0.05$ ($p = 0.001$). After that, the data was continued with LSD test.

Group	X±SD Day 3	X±SD Day 7
5%	$16.96^{b}±2.21$	$20.76^{b}±2.25$
10%	$20.83^{a}±2.13$	$24.33^{a}±2.16$
K	$16.83^{b}±2.12$	$20.16^{b}±2.78$

Note: Different Superscript Showed Significance Diference ($α < 0.05$).

Table 1. Mean Expression of BMP-2 in Treatment Group and Control Group.

Group	X±SD Day 7
5%	$18.16^{b}±2.02$
10%	$24.00^{a}±2.04$
K	$17.00^{b}±2.19$

Note: Different Superscript Showed Significance Diference ($α < 0.05$).

Table 2. Mean Number of Osteoblasts in Treatment Group and Control Group.

Post Hoc test showed there is no significant difference in expression of BMP-2 and Osteoblast between control group and 5% gel, but the comparison between 10% gel group with two other groups shows the significant difference in expression of BMP-2 and number of Osteoblasts by the 3rd and 7th day.

Discussion

Socket post extraction make a clot from blood product forms in the area and the inflammatory response. The epithelial cells at the periphery of the injury will lose their desmosomal intercellular junction and migrate to form a new epithelial surface layer beneath the clot. It is very important in repair of the connective tissue and must be retained in the first day of repair because it acts as a guide to form a new surface. After the epithelial surface is repaired, the clot is broken down by enzymes because it is no longer needed. Repair of the epithelium is tied to the repair-taking place in the deeper connective tissue.[10]

Bone morphogenetic protein-2 plays a role in bone tissue formation directly by intramembranous ossification through differentiation of mesenchymal cells into osteoblasts. Intramembranous ossification is a process of bone tissue formation directly from progenitor cells without being preceded by stages of cartilage tissue formation. The mandible is a bone formed through intramembranous ossification. Formation begins with the presence of mesenchymal cells in the area of alveolar bone repair. The area of bone to be repaired undergoes vascularization and mesenchymal cells differentiate into osteoprogenitor cells. Cytoplasm osteoprogenitor cells undergo changes from eosinophilic to basophilic, changes also occur in the golgi body which becomes clearer, this change occurs in the process of differentiation into osteoblasts which secrete collagen tissue, bone sialoprotein, osteocalcin, and bone/osteoid matrix components.

Osteoblasts and bone matrix will increase in number and bind to the cytoplasm, then the bone matrix calcifies and the relationship between the cytoplasmic protrusion of bone-forming cells will become osteocytes with canaliculosis.[11-12]

The result showed increasing expression BMP-2 by the 3th day because of the response of the wound area after tooth extraction experienced a faster improvement in new bone formation through the cellular mechanism of BMP-2 formation. BMP-2 expression on day 3 was dominated by mesenchymal cells around the alveolar bone after tooth extraction. BMP-2 is a major factor in the differentiation of osteoblasts during physiological processes and reparative osteogenesis, and the increase in expression in the treatment group showed the formation of alveolar bone tissue that was faster in the mandible after tooth extraction. Research conducted by Tseng et al. (2011) found BMP-2 expression increased in osteoblasts through the role of HIF-1α. The same thing is expressed by Zou et al. (2011) who used gene therapy in experimental animal models, found that HIF-1α was able to increase BMP-2, contribute to osteogenesis, angiogenesis and increase calvarial bone volume in mice. BMP-2 expression in the 7th day alveolar socket after extracting showed the response of the wound area after tooth extraction experienced a faster improvement in new bone formation through cellular mechanism of BMP-2 protein formation until the 7th day. BMP-2 expression on day 7 was dominated by osteoblasts. The process of differentiating osteoblasts through BMP ligands binds to receptors, 2 pairs of BMPR-I and BMPR-II form a heterotetrameric-activated receptor complex protein Smad which is a substrate of BMPR-I and plays a role in conveying signals from respectors to the target gene in the cell nucleus. Dimeric ligand bonds to heterotetrameric BMP receptors activate intrinsic serine/threonine kinase activity and subsequently R-Smad phosphorylate. BMP-2 can phosphorylate intracellular transducers, Smad 1 and 5, which causes osteoblast cell differentiation.[4,13]

The number of osteoblasts increased in the alveolar socket on the 7th day after 10% extraction due to the treatment group had alveolar bone repair after tooth extraction, through the role of BMP-2 which caused proliferation of mesenchymal cells to differentiate into active osteoblast cells more quickly. This is supported by previous studies that found the role of BMP-2 is able to cause differentiation of mesenchymal cells into osteoblasts and cause new bone formation more quickly.[14-15] BMP-2 plays a role in osteogenesis through differentiation of mesenchymal cells into osteoblasts. BMP-2 can phosphorylate intracellular transducers, Smad 1 and 5, which cause osteoblast cell differentiation (Tseng *et al*., 2010). According to Shahabooei et al. (2015) on day 7, inflammatory cells will decrease in number, blood vessel formation decreases, and osteoblasts appear active around the edges of new bone. The formation of the new alveolar bone, starting from the apical and lateral wall of the tooth extraction socket leads to the middle socket.

Conclusion

In conclusion, the application of 10% *A. cordifolia* (Ten.) Steenis gel can accelerate the optimum escalation expression of BMP-2 and the number of Osteblasts cell post tooth extraction in Wistar Rats.

Declaration of Interest

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